

*Plant Molecular Biology and Biotechnology*, B.R. Glick and J.E. Thompson, eds., CRC Press, Boca Raton, Florida (1993); P.F. Smith-Keary, *Molecular Genetics of Escherichia coli*, The Guilford Press, New York, NY (1989); *Plasmids: A Practical Approach*, 2nd Edition, Hardy, K.D., ed., Oxford University Press, New York, NY (1993); *Vectors: Essential Data*, Gacesa, P., and Ramji, D.P., eds., John Wiley & Sons Pub., New York, NY (1994); *Guide to Electroporation and electrofusions*, Chang, D., et al., eds., Academic Press, San Diego, CA (1992); *Promiscuous Plasmids of Gram-Negative Bacteria*, Thomas, C.M., ed., Academic Press, London (1989); *The Biology of Plasmids*, Summers, D.K., Blackwell Science, Cambridge, MA (1996); *Understanding DNA and Gene Cloning: A Guide for the Curious*, Drlica, K., ed., John Wiley and Sons Pub., New York, NY (1997); *Vectors: A Survey of Molecular Cloning Vectors and Their Uses*, Rodriguez, R.L., et al., eds., Butterworth, Boston, MA (1988); *Bacterial Conjugation*, Clewell, D.B., ed., Plenum Press, New York, NY (1993); Del Solar, G., et al., "Replication and control of circular bacterial plasmids," *Microbiol. Mol. Biol. Rev.* 62:434-464 (1998); Meijer, W.J., et al., "Rolling-circle plasmids from *Bacillus subtilis*: complete nucleotide sequences and analyses of genes of pTA1015, pTA1040, pTA1050 and pTA1060, and comparisons with related plasmids from gram-positive bacteria," *FEMS Microbiol. Rev.* 21:337-368 (1998); Khan, S.A., "Rolling-circle replication of bacterial plasmids," *Microbiol. Mol. Biol. Rev.* 61:442-455 (1997); Baker, R.L., "Protein expression using ubiquitin fusion and cleavage," *Curr. Opin. Biotechnol.* 7:541-546 (1996); Makrides, S.C., "Strategies for achieving high-level expression of genes in *Escherichia coli*," *Microbiol. Rev.* 60:512-538 (1996); Alonso, J.C., et al., "Site-specific recombination in gram-positive theta-replicating plasmids," *FEMS Microbiol. Lett.* 142:1-10 (1996); Miroux, B., et al., "Over-production of protein in *Escherichia coli*: mutant hosts that allow synthesis of some membrane protein and globular protein at high levels," *J. Mol. Biol.* 260:289-298 (1996); Kurland, C.G., and Dong, H., "Bacterial growth inhibited by overproduction of protein,"

*Mol. Microbiol.* 21:1-4 (1996); Saki, H., and Komano, T., "DNA replication of IncQ broad-host-range plasmids in gram-negative bacteria," *Biosci. Biotechnol. Biochem.* 60:377-382 (1996); Deb, J.K., and Nath, N., "Plasmids of corynebacteria," *FEMS Microbiol. Lett.* 175:11-20 (1999); Smith, G.P., "Filamentous phages as cloning vectors," *Biotechnol.* 10:61-83 (1988); Espinosa, M., *et al.*, "Plasmid rolling circle replication and its control," *FEMS Microbiol. Lett.* 130:111-120 (1995); Lanka, E., and Wilkins, B.M., "DNA processing reaction in bacterial conjugation," *Ann. Rev. Biochem.* 64:141-169 (1995); Dreiseikelmann, B., "Translocation of DNA across bacterial membranes," *Microbiol. Rev.* 58:293-316 (1994); Nordstrom, K., and Wagner, E.G., "Kinetic aspects of control of plasmid replication by antisense RNA," *Trends Biochem. Sci.* 19:294-300 (1994); Frost, L.S., *et al.*, "Analysis of the sequence gene products of the transfer region of the F sex factor," *Microbiol. Rev.* 58:162-210 (1994); Drury, L., "Transformation of bacteria by electroporation," *Methods Mol. Biol.* 58:249-256 (1996); Dower, W.J., "Electroporation of bacteria: a general approach to genetic transformation," *Genet. Eng.* 12:275-295 (1990); Na, S., *et al.*, "The factors affecting transformation efficiency of coryneform bacteria by electroporation," *Chin. J. Biotechnol.* 11:193-198 (1995); Pansegrau, W., "Covalent association of the *traI* gene product of plasmid RP4 with the 5'-terminal nucleotide at the relaxation nick site," *J. Biol. Chem.* 265:10637-10644 (1990); and Bailey, J.E., "Host-vector interactions in *Escherichia coli*," *Adv. Biochem. Eng. Biotechnol.* 48:29-52 (1993).

## EXAMPLES

[0049] The following examples are illustrative only and are not intended to limit the scope of the invention as defined by the appended claims.

### Strains and Media

[0050] Bacterial strains used were *Corynebacterium glutamicum* ATCC 21253 and NRRL B-11474. These strains have an auxotrophy for homoserine (ATCC 21253) and for threonine, methionine and alanine (NRRL B-11474).

[0051] Defined medium for *Corynebacterium glutamicum* ATCC 21253 contained the following ingredients (per liter): glucose, 20 g; NaCl, 2 g; citrate (trisodium salt, dihydrate), 3 g;  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.1 g;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.5 g;  $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$ , 75 mg;  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 50 mg; 100x salt solution, 20 ml;  $\text{K}_2\text{HPO}_4$ , 4 g;  $\text{KH}_2\text{PO}_4$ , 2 g;  $(\text{NH}_4)_2\text{SO}_4$ , 7.5 g; urea, 3.75 g; leucine, 0.1 g; threonine, 0.15 g; methionine, 0.05 g; thiamine, 0.45 mg; biotin, 0.45 mg; pantothenic acid, 4.5 mg (pH 7.0). The salt solution contained the following ingredients (per liter):  $\text{MnSO}_4$ , 200 mg;  $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ , 20 mg;  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ , 10 mg;  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ , 200 mg;  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 50 mg;  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ , 20 mg (pH 2.0).

[0052] Defined medium for *Corynebacterium glutamicum* NRRL B-11474 contained the following ingredients (per liter): glucose, 20 g; NaCl, 1 g,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.4 g;  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.01 g;  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ , 0.01 g;  $\text{KH}_2\text{PO}_4$ , 1 g;  $(\text{NH}_4)_2\text{SO}_4$ , 10 g; urea, 2.5 g; alanine, 0.5 g; threonine, 0.25 g; methionine, 0.5 g; thiamine, 0.45 mg; biotin, 0.45 mg; niacinamide, 50 mg (pH 7.2).

[0053] *Pyruvate Carboxylase and Phosphoenol Pyruvate Carboxylase Assay*

[0054] Pyruvate carboxylate and phosphoenol pyruvate carboxylate assays were performed with permeabilized cells prepared by the following method. Log phase cells were harvested by centrifugation for 10 min at 5000 xg at 4°C and washed